Finally, the new results raise a number of questions about the mechanisms by which clonally related ensembles are formed. What molecular cues and physical forces are responsible for the various movements involved, such as migration of the IPs, neurons, and astrocytes along the RGCs, bending of the RGCs, and separation of the RGCs and the neurons as the latter mature into full-blown pyramidal neurons? Not only will the answers to these questions illuminate the molecular mechanisms by which neuronal ensembles (and possibly cell assemblies) are formed, but they may provide clues to the origins of diseases in which miswiring is known to play a central role, including autism and schizophrenia.

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The Angiotensin II Type 2 Receptor for Pain Control

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All well-known deleterious effects of angiotensin (Ang) II, including vasoconstriction, inflammation, water and salt retention, and vascular remodeling, are mediated via its type 1 (AT_1) receptor. This explains why AT_1 receptor blockers (ARBs) and inhibitors of Ang II synthesis, such as ACE inhibitors and renin inhibitors, are beneficial for cardiovascular disease. Yet, Ang II has a second receptor, the Ang II type 2 (AT_2) receptor, the function of which, even after over 20 years of research, remains largely unknown. In this issue, Marion et al. provide a new chapter to the AT_2 receptor story.

Previously, it was proposed that Ang II type 2 (AT_2) receptors antagonize the effect of the AT_1 receptor, and that the beneficial effects of ARBs could be due to the stimulation of the unoccupied AT_2 receptor. However, subsequent studies revealed that AT_2 receptors are not always protective (Verdonk et al., 2012a), and the AT_2 receptor agonist C21, at AT_2 receptor-selective doses, does not lower blood pressure (Verdonk et al., 2012b). Of interest, AT_2 receptors were shown to stimulate neurite outgrowth, and AT_2 inhibitors to reduce pain signaling in animal models and in rodent and human sensory neurons in vitro (Anand et al., 2013) (Figure 1A). As such, they are potential targets for agonists in nerve regeneration and for antagonists to suppress pain. Recently, the latter concept has been successfully tested in patients with postherpetic neuralgia, where the AT_2 receptor antagonist EMA401 in a randomized, double-blind, placebo-controlled phase 2 trial significantly reduces pain over 4 weeks of treatment (Rice et al., 2014). This is the only human trial so far involving drugs acting on AT_2 receptors, which suggests that AT_2 receptor antagonists might be further developed to treat neuropathic pain.

In this issue of *Cell*, Marion et al. (2014) now suggest that AT_2 receptor stimulation may induce analgesia. Following the clinical observation that *Mycobacterium ulcerans*, the etiological agent of Buruli ulcer, causes extensive skin lesions that are not accompanied by pain, they study a mouse footpad model in which pain

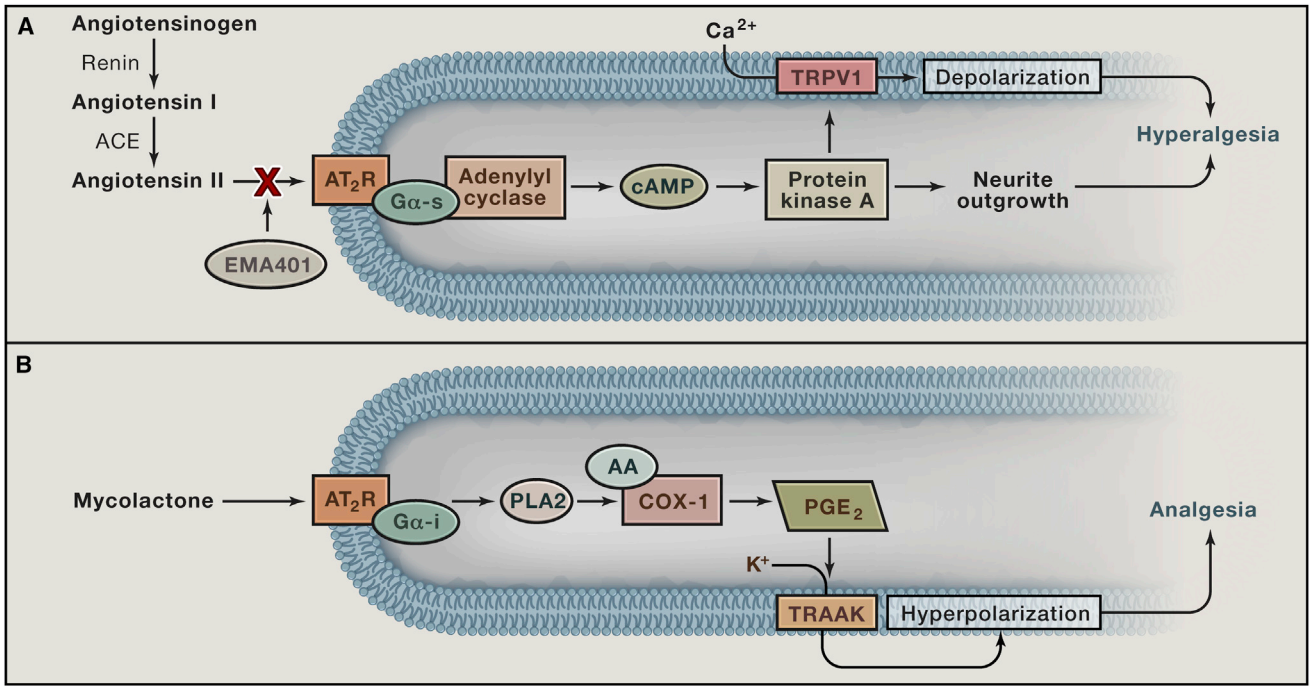

Figure 1. Angiotensin II Formation and the Subsequent Activation of AT_2 Receptors, Resulting in Either Hyperalgesia or Analgesia
(A) The hyperalgesia pathway predominates in peripheral primary nociceptors and converges with the nerve growth factor-TRPV1 (transient receptor potential vanilloid subfamily member 1) pain pathway.
(B) The mycolactone analgesia pathway may predominate in inflammatory cells and central nervous system neurons. PLA2, phospholipase A2; AA, arachidonic acid; COX-1, cyclooxygenase-1; PGE_2, prostaglandin E_2; TRAAK, TWIK-related, arachidonic acid-activated K^+ channel.

responses are diminished, but the proximal nerve shows no damage. Next, the authors screen a siRNA library targeting 8,000 host genes, making use of macrophage-like cells of the Raw267.4 cell line. They monitor cellular hyperpolarization following exposure to mycolactone, the main toxin secreted by *M. ulcerans*. Unexpectedly, this screening only yields three receptors, of which the AT_2 receptor appears the most promising. Subsequently, with the help of AT_2 receptor knockout mice and the AT_2 receptor antagonist PD123319, the authors demonstrate a potential role for this receptor in nociception and the molecular mechanism involved (Figure 1B). Mycolactone, at micromolar levels, is found to bind both AT_1 and AT_2 receptors, but can only activate the AT_2 receptor. This activation is Gαi-dependent and leads to phospholipase A2-mediated arachidonic acid (AA) liberation and the generation of prostaglandin E2 (PGE_2) from AA by cyclooxygenase-1 (COX-1). PGE_2 can cause neuronal hyperpolarization through activation of the KCNK4 (TRAAK) K^+ channel, thereby inducing analgesia.

These findings, although exciting, raise a number of questions. First, they oppose the current view, supported by clinical evidence, that AT_2 receptor antagonism and COX inhibition suppress pain, and that COX-2, rather than COX-1, is upregulated in inflammatory conditions. In fact, the data from mice infected with *M. ulcerans* and treated with the COX inhibitor piroxicam further suggest that classical pain suppressants like NSAIDs (which block COX) would enhance pain in patients with Buruli ulcer. Furthermore, TRAAK activation by PGE_2, rather than AA, is unusual (Noël et al., 2011), and the observation that millimolar concentrations of the K^+ channel blockers tetraethylammonium and $BaCl_2$ prevent hyperpolarization is at most suggestive for TRAAK involvement, but not conclusive.

It should be mentioned that the molecular studies are performed in neonatal mouse hippocampal neurons, pheochromocytoma cell 12 (PC12) cells, and macrophage-like cells, but not in intact animals or sensory neurons/nociceptors, leaving open the question of where exactly the AT_2 receptor activation occurs in the footpad after *M. ulcerans*/mycolactone injection. Surprisingly, there is no alteration in footpad AT_2 receptor density following infection. One alternative explanation might be that these AT_2 receptors are not neuronal, but located on non-neuronal cells, such as macrophages or fibroblasts, where they might exert anti-inflammatory effects, such as suppressing the release of cytokines (Rompe et al., 2010). In addition, mycolactone-dependent inhibition of protein translocation into the endoplasmic reticulum may also explain the deficit of cytokines (Hall et al., 2014). Such effects would explain the lack of inflammation, and, thereby, the lack of inflammatory pain sensation. Previous animal model studies have reported transient early hyperesthesia and then hypoesthesia with nerve damage. The latter is also observed in clinical skin biopsies (Zavattaro et al., 2012), although these published studies have not used gold-standard methods to assess nociceptors or pain receptors in affected cutaneous tissues. In this regard, it is of interest to note that the cutaneous loss of nociception in leprosy, also caused by a

Mycobacterium (*M. leprae*), involves loss of intraepidermal nerve fibers and pain receptors in clinical skin biopsies (Facer et al., 2000). The absence of inflammatory pain may thus result from effects of mycolactone on both inflammatory cells and cutaneous nociceptors.

The mycolactone concentrations required to stimulate AT_2 receptors are in the 3 μg/ml range (~4 μmol/l), whereas the level required to cause hyperpolarization in vitro and that in the tissue after infection are one to two orders of magnitude lower. Clearly, more evidence is needed to confirm that mycolactone truly is a bona fide AT_2 receptor agonist at relevant in vivo levels. It is also important to know the exact location of the AT_2 receptors, and why mycolactone, despite binding to AT_1 receptors, does not stimulate these receptors. Studies with well-established AT_2 receptor agonists like C21 and clinicopathological correlations may help to answer these questions.

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Designer Proteins to Trigger Cell Death

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Efforts to generate biologically active proteins by de novo computational design have been limited to creating functional sites within pre-existing scaffolds. Procko et al. use an innovative computational design approach coupled with in-vitro-targeted evolution to produce a potent polypeptide inhibitor of a viral Bcl-2-like protein. This novel inhibitor triggers apoptosis of virus-infected cells.

Metazoan organisms employ a distinct type of programmed cell death, called apoptosis, to eliminate severely damaged or infected cells. The Bcl-2 gene family encodes proteins that control apoptotic signaling via the cell-intrinsic, mitochondrial pathway (Cory and Adams, 2002). Two structural subclasses of this family share Bcl-2 homology (BH) motifs: (1) BH3 proteins (e.g., Bim, Bid, Bad, Bmf, Puma, and Noxa), which harbor a single BH motif and promote apoptosis; and (2) multi-BH proteins, which possess three or four BH regions and act either as apoptosis activators (e.g., Bax and Bak) or inhibitors (e.g., Bcl-2, Bcl-x_L, Bcl-w, and Mcl-1). Some BH3 factors promote apoptosis by sequestering prosurvival Bcl-2 proteins from Bax and Bak, whereas others bind directly to Bax or Bak to drive activation. The BH3 motif—an ~26 amino acid α-helical peptide—interacts with a hydrophobic groove on the cognate binding partner.

Viruses often encode orthologs of cellular antiapoptotic proteins to prevent host cell death and extend viral replication. Epstein-Barr virus (EBV) latently infects human B cells, contributing to cancers such as Burkitt's lymphoma.